Amer. Malac. Bull. **32**(2): 290–307 (2014) http://www.bioone.org/toc/malb/32/2

Exploring the diversity of mesopsammic gastropods: How to collect, identify, and delimitate small and elusive sea slugs?*

Katharina M. Jörger, Timea P. Neusser, Bastian Brenzinger, and Michael Schrödl

Mollusca Department, SNSB-Bavarian State Collection of Zoology, Münchhausenstr. 21, 81247 München, Germany and Department Biology II, BioZentrum, Ludwig-Maximilians-Universität, Großhadernerstr. 2, 82152 Planegg-Martinsried, Germany
Correspondence, Katharina Jörger: Katharina.Joerger@zsm.mwn.de

Abstract: Sediment-covered ocean floors constitute one of the largest and at the same time least explored habitats on Earth, still hiding an unknown level of species diversity. Coastal areas of this marine mesopsammic habitat harbor a variety of heterobranch snails and slugs. These gastropods long were puzzling due to their unclear phylogenetic positions, their aberrant morphologies and the lack of knowledge regarding their biology and diversity. Herein, we briefly review the advances of interstitial gastropod exploration, emphasizing that molecular approaches on formerly enigmatic mesopsammic groups like rhodopemorphs or acochlidians contributed to a drastic reconsideration of heterobranch systematics and evolution. We give an overview of the known diversity of mesopsammic heterobranchs and a list of type localities. In order to enhance surveys on the biodiversity of yet unexplored coasts, we then provide a suitable method to take samples of mesopsammic heterobranchs, and to extract and document slugs and snails from sands. A key based largely on externally-visible features allows for initial identification of already known taxa. Most mesopsammic gastropods show a "meiofaunal syndrome", *i.e.*, their morphology is constrained by the spatially-restricted interstitial environment, favoring rather uniform, worm-like body shapes and simple internal organization, which causes problems in conventional taxonomic approaches. Here, we present and discuss an integrative taxonomic workflow for delimiting potentially cryptic and elusive mesopsammic species, that also may be of use for other rare(ly) sampled invertebrates.

Key words: Acochlidia, field key, integrative taxonomy, Mollusca, Panpulmonata

THE MESOPSAMMON AND ITS INHABITANTS

Marine sediments and the interstices between sand grains, sometimes referred to as mesopsammon (Remane 1940), belong to the most ancient ecosystems of our planet (Rundell and Leander 2010). By the mid-19th and at the beginning of the 20th century, scientists discovered the water-filled interstitial space between the grains of coastal marine sands as a habitat for organisms (*e.g.*, Lovén 1844, Kowalevsky 1901a, 1901b, Giard 1904). Considerable progress has yet been achieved in different areas of meiofaunal research (*e.g.*, Remane 1952, Swedmark 1964, Ax 1969, Higgins and Thiel 1988a, Worsaae and Kristensen 2005, Giere 2009, Curini-Galletti *et al.* 2012, Worsaae *et al.* 2012). However, our knowledge of meiofaunal biodiversity, ecology and evolution is still limited and Rundell and Leander (2010) emphasized that the exploration of the meiofauna "remains among the most challenging, the most neglected and potentially the most enlightening frontiers of discovery in biology".

The interstitial milieu is characterized by extreme ecological conditions, such as faint light and limited amount of space, which restricts the body size and limits the interstitial fauna to minute, vermiform organisms suited to a lacunar environment (Swedmark 1964, 1968, Ax 1969, Higgins and Thiel 1988a). Currents and wave action transform the interstitial biotope by permanent restratification of the surface layer of the sand (Swedmark 1964). The continuous rearrangement of the particles contributes to a dynamic environment and makes the colonization by, *e.g.*, algae, difficult (Swedmark 1968). Furthermore, the living conditions in the intertidal zone or shallow water are complicated by diverse physical factors: the temperature varies with the time of day, seasons, and the rhythm of tides and, thus, fluctuates significantly in the surface layers of the sand layer; and, the salinity may increase by evaporation or decrease by rainfall or by the inflow of coastal freshwater (Giere *et al.* 1988). Organisms that successfully colonize the marine interstitial often develop special morphological and biological adaptations: body sizes are typically very small ranging from 0.5 mm to approx. 3 mm; flat

*From the "Mollusks: Magnitude of molluscan diversity – the known and the unknown" Symposium held at the 78th meeting of the American Malacological Society, Cherry Hill, New Jersey, June 19–20, 2012. Symposium manuscripts were reviewed and accepted by the Symposium Organizer and Guest Editor, Dr. Ira Richling.

and broad or vermiform-elongated body shapes are commonly favored. The body wall is often reinforced by subepidermal spicules or cuticle for mechanical protection. Members of the interstitial fauna frequently have a strong contractibility and a high adhesive capability by different glandular systems to avoid being washed away (Swedmark 1964, 1968, Botosaneanu 1986, Higgins and Thiel 1988a). Consequently, the study of mesopsammic taxa is challenging—species are small, hard to collect, problematic to distinguish externally and difficult to describe by means of traditional techniques.

Different terms are associated with the fauna inhabiting marine sediments (see Higgins and Thiel 1988b, for summary). Most commonly a practical size definition is applied, characterizing all fauna which passes through a 1 mm mesh and is retained by 42 μm mesh as meiofauna (Higgins and Thiel 1988b). While this definition provides no relationship to a specific ecology and is also controversial due to deviations depending on *e.g.*, anesthetized vs. living organisms, it is still of high practical value as directly related to extraction techniques in the field (Higgins and Thiel 1988b). Nearly all major metazoan taxa are represented in this size-defined marine meiofauna, *e.g.*, Cnidaria, Platyhelminthes, Nemertea, Gnathostomulida, Gastrotricha, Rotifera, Annelida, Priapulida, Loricifera, Kinorhyncha, Acari, crustacean taxa, and Mollusca (*e.g.*, Swedmark 1964, Botosaneanu 1986, Higgins and Thiel 1988a, Giere 2009, Rundell and Leander 2010). With the exception of Cephalopoda, all major molluscan clades include members that are at least temporarily meiofaunal (*i.e.*, in early stages of their life cycle). Juveniles of molluscan taxa that inhabit soft sediments as adults (as sand-dwellers or epibenthic) are frequently encountered in sediment samples. Infaunal Scaphopoda and sediment-burrowing Caudofoveata are temporarily meiofaunal; few prochaetodermatid Caudofoveata retain meiofaunal sizes as adults (Morse and Scheltema 1988). The same applies for several Polyplacophora (pers. obs.; E. Schwabe pers. comm.), with currently only one species—*Leptochiton intermedius* (Salvini-Plawen, 1968)—being described as permanent meiofauna (Salvini-Plawen 1968). Among infaunal Bivalvia and Solenogastres, however, several representatives are known with permanently minute body sizes (*e.g.*, bivalve Nuculidae and Mallettiidae (Poizat and Arnaud 1988) or neomeniomorph Meiomenidae and Simrothiellidae (Morse and Scheltema 1988, García-Alvarez *et al.* 2000)). Among gastropod molluscs, we also frequently encounter temporarily meiofaunal forms in marine sediment samples. For example representatives of cephalaspidean *Chelidonura* A. Adams, 1850, nudibranchs *Gymnodoris* Stimpson, 1855, *Aegires* Lovén, 1844 and some chromodoridids, or some Runcinacea (*Runcina* Forbes [in Forbes and Hanley], 1851) were repeatedly observed in sand samples (pers. obs.). There are also several clades of shelled gastropods (*e.g.*, Pyramidellidae, Omalogyridae, Caecidae, Neritiliidae, Seguenziidae) with minute body sizes assigning them to permanent meiofauna. In most of these cases it is unknown, however, whether these snails lead an epibenthic or infaunal lifestyle.

The fauna inhabiting the interstices of sediment grains and which moves through its habitat with minimal disturbance (*i.e.*, in contrast to organisms digging through the sand) is defined as 'interstitial' (Nicholls 1935) or 'mesopsammic' (Remane 1940) fauna. Usually this mesopsammic or interstitial fauna also falls in the size-defined category of meiofauna but not necessarily so when inhabiting very coarse sediments and shell gravel. Some 'typical' meiofaunal snails (*sensu* Arnaud *et al.* 1986) that are frequently extracted from bulk sediment samples may not be mesopsammic; instead they are surface dwellers that never venture deep into the sand (*e.g.*, most *Caecum* Fleming, 1813 or *Embletonia* Alder and Hancock, 1851), or venture across the sand but live mostly on algae (*Omalogyra* Jeffreys, 1859, Runcinacea, large *Rhodope* Koelliker, 1847). In the present review we focus on meiofaunal and at least externally shell-less gastropods that show the characteristic adaptations of their body plan typical for interstitial taxa (see above) and which can, therefore, be considered as truly inhabiting the marine mesopsammon.

MESOPSAMMIC SLUGS PLACED IN A PHYLOGENY

Mesopsammic heterobranchs from different lineages (Fig. 1) often look quite similar to other meiofaunal "worms", having streamlined vermiform bodies and often lacking tentacles, body appendages or pigments, and even anatomically show similar organ reductions or special structures such as accessory ganglia or spicules. This phenomenon of particular morphoanatomical similarity caused by adaptation to a special environment was termed the "meiofaunal syndrome" by Brenzinger, Haszprunar *et al.* (2013). Multiple convergence in virtually all major organ systems causes problems not only for species identification and assignment of aberrant worm-like species to higher taxa, but especially for reconstructing their relationships in morphocladistic analyses (Schrödl and Neusser 2010).

Therefore, trials to recover the origin of mesopsammic heterobranch lineages using multi-locus sequence data appeared more promising and have, in hindsight, given invaluable contributions to heterobranch systematics. Vonnemann *et al.* (2005) first included three acochlidian species (representing both major subclades) into molecular analyses (18S and 28S rRNA), and failed to recover monophyletic opisthobranchs or pulmonates. Expanding the heterobranch taxon sampling and adding mitochondrial COI and 16S rRNA markers, Klussmann-Kolb *et al.* (2008) recovered a tree that

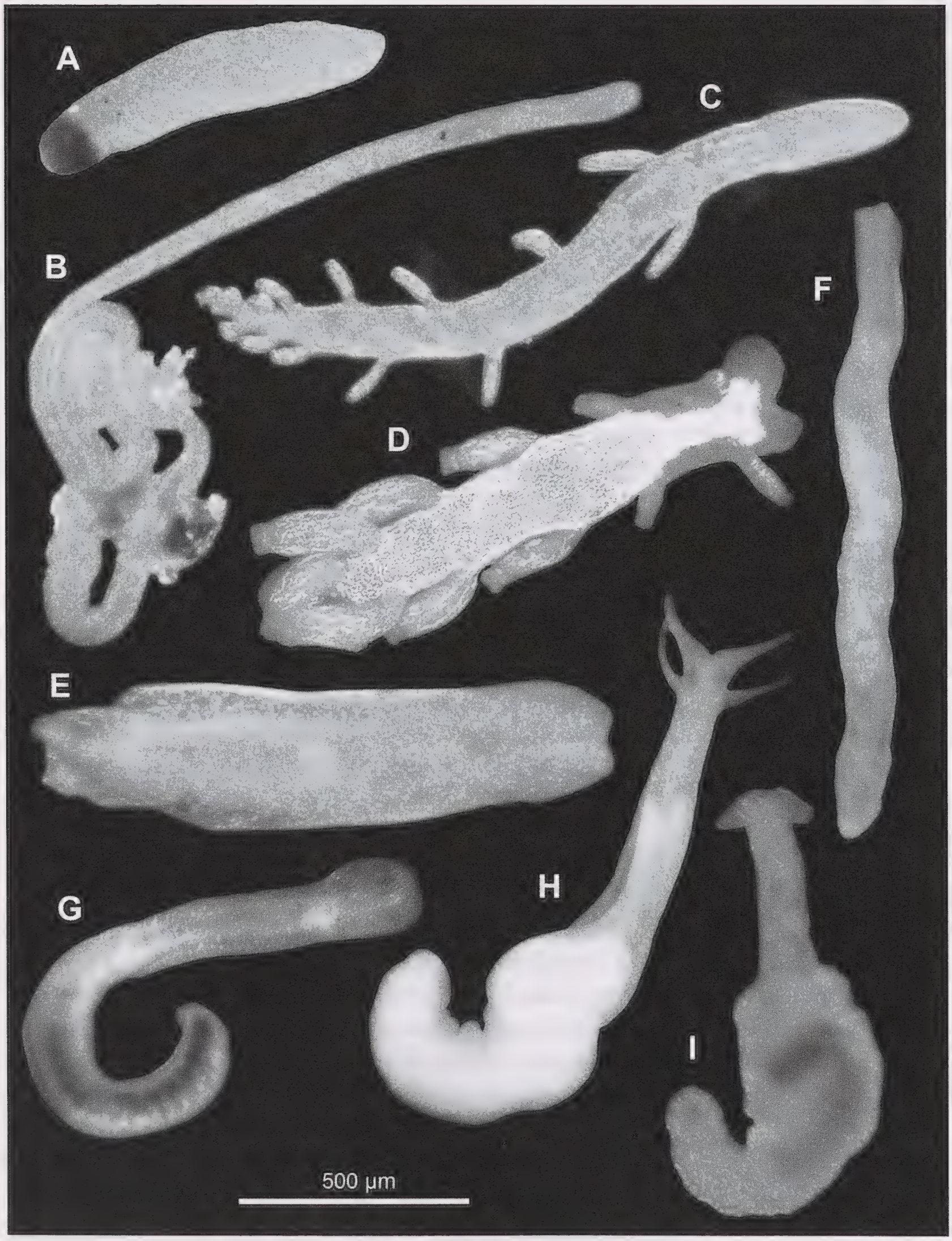

Figure 1. Living specimens of the major mesopsammic slug lineages. **A,** *Rhodope* sp. from Belize (Rhodopidae, Rhodopemorpha); **B,** *Helminthope* sp. from Papua (Rhodopidae, Rhodopemorpha); **C,** *Pseudovermis salamandrops* (Pseudovermidae, Aeolidoidea?); **D,** *Embletonia pulchra* (Embletoniidae, Aeolidoidea?); **E,** *Philine exigua* ('Philinidae', Cephalaspidea); **F,** *Philinoglossa marcusi* (Philinoglossidae, Cephalaspidea); **G,** *Platyhedyle denudata* (Platyhedylidae, Sacoglossa); **H,** *Pseudunela viatoris* (Pseudunelidae, Acochlidia); **I,** *Pontohedyle milaschewitchii* (Microhedylidae, Acochlidia). (Color shown in electronic version only).

clearly contradicted Milne Edwards' (1848) classical division of the Euthyneura into Opisthobranchia and Pulmonata. Jörger, Stöger *et al.* (2010) reviewed potential morphological evidence for Pulmonata or Opisthobranchia, and could not find any. Retrospectively, Opisthobranchia or Pulmonata have never been well-supported monophyla (Haszprunar 1985, Dayrat and Tillier 2002), but the scientific community has long kept to a traditional concept of the two euthyneuran subtaxa, particularly because it conveniently bears a rough resemblance to the ecological division between sea slugs on the one hand and limnic and terrestrial slugs and snails on the other (Wägele *et al.* 2014). Based on multilocus data on a heterobranch taxon sampling including mesopsammic Philinidae, Philinoglossidae, Platyhedylidae, and six of seven acochlidian families, Jörger, Stöger *et al.* (2010) formally reclassified the Euthyneura. Major novelties were the exclusion of Acteonoidea, and the basal position of Nudipleura (with unsampled mesopsammic Pseudovermidae and Embletoniidae) sister to a clade with all other euthyneurans, termed Tectipleura (Schrödl *et al.* 2011). The latter divides into Euopisthobranchia, including Cephalaspidea *sensu stricto* with interstitial philinids and philinoglossids, and Panpulmonata, comprising sacoglossans (with mesopsammic *Platyhedyle* Salvini-Plawen, 1973) and Acochlidia related to siphonariids, pyramidellids, glacidorbids, amphibolids, and typical pulmonate groups (Fig. 2). Mesopsammic, extremely worm-like rhodopemorphs (Brenzinger, Wilson *et al.* 2011, Brenzinger, Haszprunar *et al.* 2013) surprisingly clustered with shelled and long-spired Murchisonellidae, which are basal heterobranchs (Brenzinger *et al.* 2014) in initial molecular analyses (Wilson *et al.* 2010). The new heterobranch tree is shown and discussed by Wägele *et al.* (2014).

From the perspective of interstitial fauna, heterobranchs invaded the mesopsammon at least eight times independently (Fig. 2), and in conclusion adapted to the environment convergently. Interestingly, and so far unique among interstitial gastropods, several acochlidian lineages reversed 'regressive evolution' characteristic for the mesopsammic fauna (Swedmark 1968; Westheide 1987) and

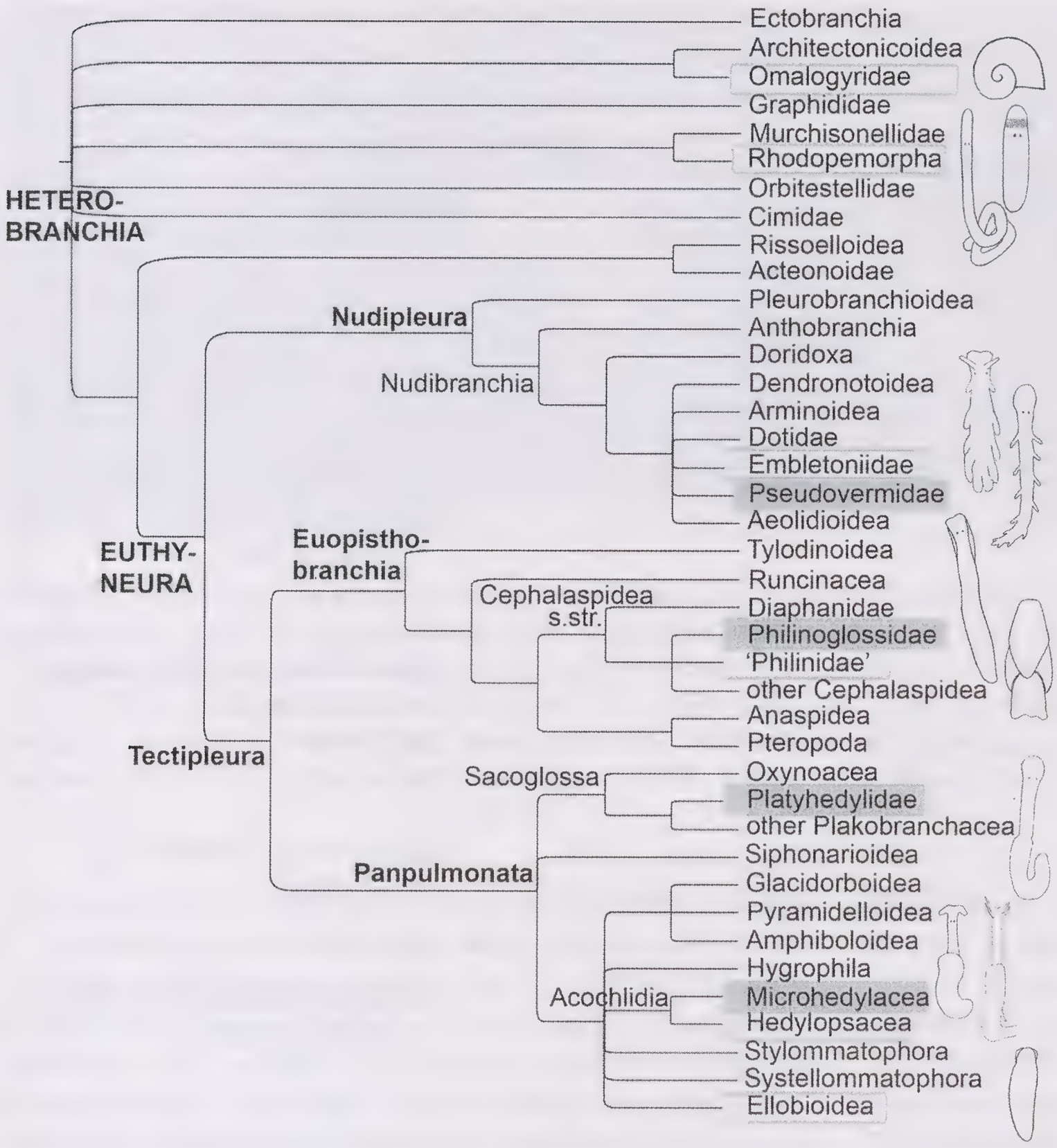

Figure 2. Cladogram of Heterobranchia modified after Jörger *et al.* (2010b), combined with the consensus topology presented in Wägele *et al.* (2014), showing mesopsammic lineages in grey boxes (clades not entirely mesopsammic with grey outline only).

reestablished an epibenthic lifestyle. Intertidal to supratidal Aitengidae adapted to semiterrestrial conditions (Neusser, Fukuda *et al.* 2011), the brackish-water adapted *Pseudunela espiritusanta* Neusser and Schrödl, 2009 lives under intertidal stones (Neusser and Schrödl 2009), and Acochlidiidae inhabit freshwater (*e.g.*, Wawra 1974, 1979, Brenzinger, Neusser *et al.* 2011).

DIVERSITY OF MESOPSAMMIC SLUGS

Compared to other meiofaunal taxa such as nematodes, polychaete annelids or copepods, all meiofaunal slug lineages are small taxonomic groups with few described species (Table 1). At current stage of research the Acochlidia are the largest clade of sea slugs inhabiting the mesopsammon with currently 26 valid species of Microhedylacea (an exclusively mesopsammic clade) and six Hedylopsacean species described from the interstices of sand grains (see Table 1, remaining Hedylopsacea live epibenthic in marine, limnic, and (semi)terrestrial systems) (Schrödl and Neusser 2010; unpublished data). Pseudovermidae are entirely mesopsammic and currently comprise 16 valid species (Urgorri *et al.* 1991, Jörger *et al.* 2014). Seven mesopsammic species of cephalaspidean Philinoglossidae are currently valid and a single species is described from the mesopsammon for cephalaspidean Philinidae and Sacoglossa (Arnaud *et al.* 1986). In Rhodopemorpha it remains unclear whether the five species of *Rhodope* truly inhabit the mesopsammon or lead an epibenthic lifestyle; the monotypic *Helmithope* is considered a truly interstitial species based on its morphological adaptations (Brenzinger, Haszprunar *et al.* 2013). Five species of Smeagolidae are reported to inhabit cobble beaches (Tillier and Ponder 1992); given the large interstitial spaces of this habitat it probably differs considerably from the mesopsammic environment. Usually, mesopsammic slugs are rare and there are only few taxa in which higher local densities (> 100 individuals / 0.05 m³) are reported (*e.g.*, some microhedylacean Acochlidia or philinoglossid Cephalaspidea (Poizat 1991)). The Acochlidia show worldwide distribution and present the only known lineage of mesopsammic sea slugs which also inhabit cold waters (*Asperspina murmanica* (Kudinskaya and Minichev, 1978) (see Neusser, Martynov *et al.* 2009)). All remaining lineages are restricted to tropical and temperate sands (Fig. 3); a compilation of type localities from all valid mesopsammic slugs is provided for future research in Appendix 1 (http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF). The comparably high diversity in European waters is likely a sampling artifact with a major effort of meiofaunal research focusing in Europe for decades (Coull and Giere 1988) and relatively little and isolated sampling effort in tropical zones (especially the Indian Ocean and large parts of the Indo-Pacific).

The sampling effort of our workgroup supported by a series of international collaborators have significantly raised the diversity in each of the lineages recovering a wealth of putative new species, which raises the number of species by up to ten fold (see Table 1). The presumably low reproductive output and dispersal abilities of meiofaunal slugs including Acochlidia suggests a high degree of endemism, which is supported by the detection of rather narrow ranges of distribution in many meiofaunal slugs and deep genetic divergence

Table 1. Status of diversity of known meiofaunal slugs (Heterobranchia). ? No data available. *According to own unpublished data. **Hedylopsacea contain at least three secondarily non-meiofaunal lineages (Aitengidae, Acochlidiidae, and *Pseudunela espiritusanta*). *Rhodope* and *Embletonia* might be epibenthic and *Smeagol* inhabits cobble beaches. Anatomical characters do not necessarily fit the 'meiofaunal syndrome'.

Taxon	Described meiofaunal species/ Estimated total number including undescribed species*	References
RHODOPEMORPHA		
Rhodope Kölliker, 1847	6 / 15*	Haszprunar and Heß 2005; Wilson *et al.* 2010
Helminthope Salvini-Plawen, 1991	1 / 8*	Brenzinger, Haszprunar *et al.* 2013
NUDIBRANCHIA		
Pseudovermis Perejaslavtzeva, 1891	16 / 20*	Urgorri *et al.* 1991
Embletonia cf. *pulchra* Alder and Hancock, 1844)	1 / 10*	Miller and Willan 1992; Martynov 2007
CEPHALASPIDEA		
Philinoglossidae	7 / 15+*	Salvini-Plawen 1973; Brenzinger, Padula *et al.* 2013
Philine exigua Challis, 1969 and similar	1 / 5-10+*	Challis 1969
SACOGLOSSA		
Platyhedyle Salvini-Plawen 1973	1 / 4+*	Salvini-Plawen 1973; Rückert *et al.* 2008
ACOCHLIDIA		
Microhedylacea	26 / 40+*	Schrödl and Neusser 2010; Jörger and Schrödl 2013
Meiofaunal Hedylopsacea**	6 / 16+*	
OTINOIDEA		
Smeagol Climo, 1980	5 / 8?	Tillier and Ponder 1992; Fukuda and Ueshima 2010

in globally distributed lineages (Jörger *et al.* 2012). Moreover, the patchy occurrence typical for meiofaunal animals (*e.g.*, Poizat and Arnaud 1988, Andrade *et al.* 2011) can easily cause species to go undiscovered even in densely sampled areas (Curini-Galletti *et al.* 2012; pers. obs.). Considering the fact that the vast majority of marine sands, worldwide and in all varying depth ranges, are still virgin soil to meiofaunal research, the currently known diversity of mesopsammic slugs and probably also the reported unpublished findings in Table 1 still severely underrepresents true diversity. Overall, the contribution of meiofauna to marine biodiversity surveys has doubtlessly been underestimated, leaving this important ecosystem largely neglected in conservation approaches. We need fast, efficient and reliable means of species delineation in meiofaunal taxa to address this taxonomic impediment—means that are capable of dealing with the putatively high degree of cryptic speciation likely to be the rule for meiofauna (Jörger *et al.* 2012). Accounting for the still exploratory stage of meiofaunal research, we aim to contribute in the following to exploring worldwide mesopsammic sea slugs by providing guidelines on how to extract specimens from sediments and a key for initial determination of mesopsammic sea slugs into major taxa at least, updating Arnaud *et al.* (1986). Moreover, we discuss the pitfalls of species delimitation in meiofaunal slugs, evaluate the pros and cons of various species delimitation methods, and propose an integrative work flow that especially addresses the needs of mesopsammic and other taxa with only few samples available.

INSTRUCTIONS TO STUDY MESOPSAMMIC SLUGS IN THE FIELD

Searching and extracting mesopsammic sea slugs

In the course of our studies we conducted sampling trips to different biogeographic zones for initial exploration of the interstitial malacofauna, and experienced very heterogeneous

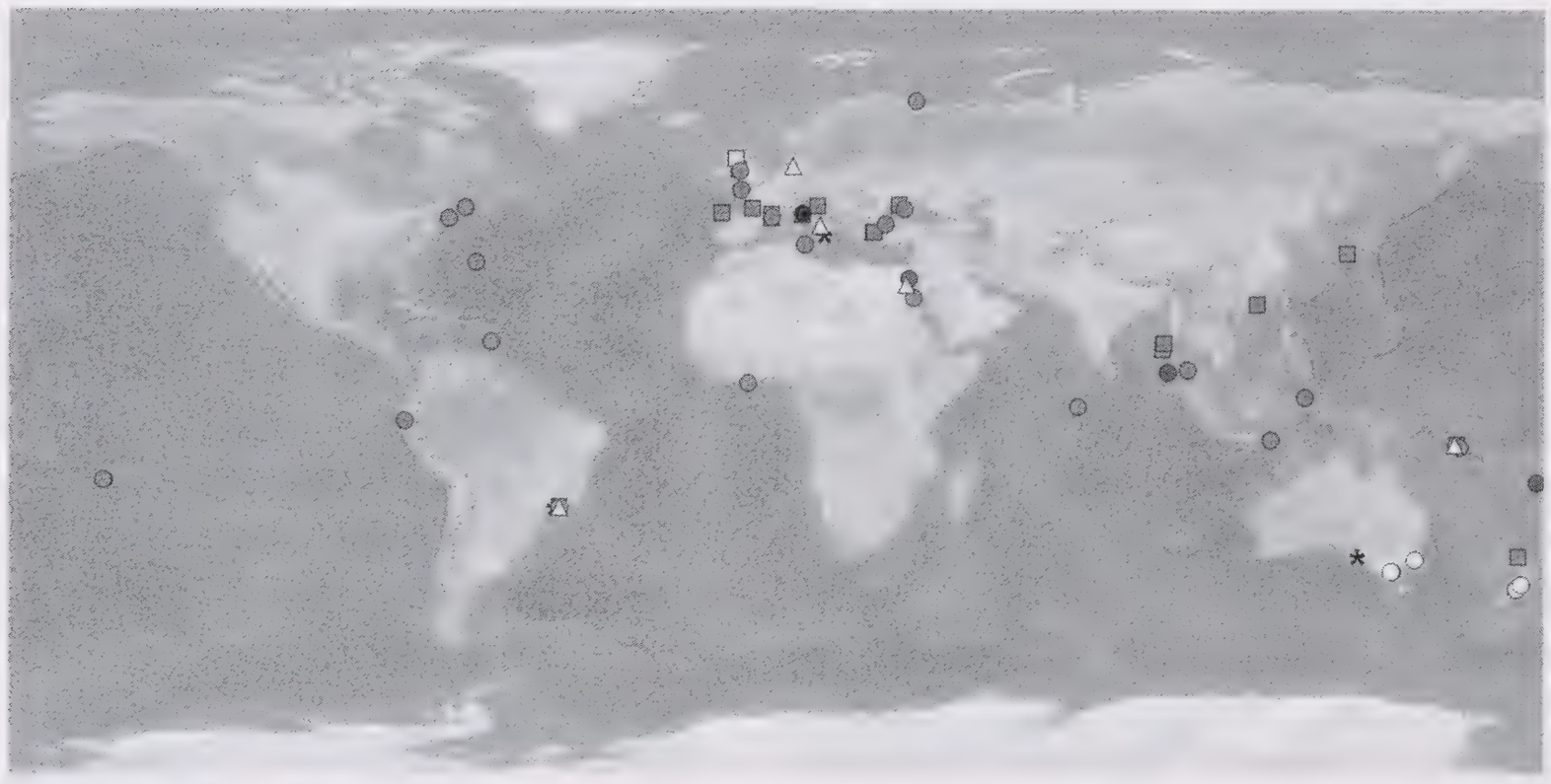

Figure 3. Overview of the type localities of the different mesopsammic microslugs (for details see also Table 1). 'Lower heterobranch' Rhodopemorpha marked with black star. Nudipleuran taxa marked with squares (dark grey: Pseudovermidae, light grey: Embletoniidae). Euopisthobranch Cephalaspidea shown in triangles (light grey: Philinoglossidae, dark grey: Philinidae). Panpulmonate clades marked as dots (dark grey: hedylopsacean Acochlidia, light grey: microhedylacean Acochlidia, white: Sacoglossa, black: Smeagolidae).

regional density of specimens and species diversity. In general, subtropical or tropical coasts seem much more species-rich than cold waters (compare in Fig. 3), and not all depths or sediment types host the same or an equally rich gastropod meiofauna. For example, in our experience lava sands are generally poorer with respect to meiofaunal gastropod diversity then coral sands. Usually, coarse oxygenated subtidal substrates with steady water currents appear privileged in species diversity relative to intertidal, wave-exposed fine sandy beaches or fine sediments with much organic content (Poizat and Arnaud 1988; pers. obs.). We now know of specialized acochlidians from all continents but Antarctica, from many islands regardless their distances to continental coasts, and even from high energy beaches or brackish water-influenced estuaries. Other mesopsammic groups such as cephalaspideans also occur in finer, detritus-rich sediments, often sporadically or seasonally (Poizat 1984). Freshwater-influenced sediments may host acochlidians and sacoglossans, and, so far, a single known acochlidian species (*Tantulum elegans* Rankin, 1979 from the Carribbean) even occurs in sediments of a swampy mountain spring (however, our own recollecting attempts at the type locality in 2009 failed).

In summary, almost all our sampling trips resulted in finding a variety of mesopsammic sea slugs and snails, often including lineages new to science (see above). To encourage and support sampling of mesopsammic slugs, we here provide a suitable and detailed step-by-step procedure to extract mesopsammic molluscs from sand samples (modified from Pfannkuche and Thiel (1988) and updated from Schrödl (2006) and Neusser (2011)) and list suggestions based on our experience on how to anesthetize and fix encountered specimens for various purposes (Appendix 2) (http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF).

Documenting mesopsammic slugs in the field

During specimen collection, researchers should consider that samples have to be treated and selected wisely, and prepared differently according to later, various uses. In absence of an external shell, the taxonomy of meiofaunal slugs or snails with reduced internal shell was mainly based on external morphology of living or preserved specimens, presence and type of calcareous spicules and radula characteristics (Kowalevsky 1901b, Arnaud *et al.* 1986, Wawra 1987). Snails and slugs may distort or retract during fixation if not relaxed very carefully, pigments may fade especially in ethanol, and calcareous spicules will disappear quickly in any calcium carbonate undersaturated or acidic fixative (Poizat and Arnaud 1988). Live documentation of ephemeral morphological features and of behavior and movement, thus, is a unique opportunity to study phenotypes and essential to preliminarily identify new findings and to correlate them with existing taxonomy. Of special interest are—next to lucky occasional observations on, *e.g.*, feeding or reproduction—the behavior during disturbance (*e.g.*, ability to retract) and the behavior in motion (*e.g.*, the ability to adhere to the substrate). Important taxonomic characters in meiofaunal slugs are the general body shape, shape and relative size of head appendages (*i.e.*, oral tentacles, rhinophores), body appendages (*i.e.*, cerata in nudibranch *Pseudovermis* and *Embletonia*), and relative length and width of the foot; all of which should preferably be documented on living material to avoid fixation artifacts. Observations of internal anatomy (*e.g.*, (different types of) spicules or pigments (including eyes)) are most informative using carefully squeezed, anesthetized specimens under transmission light microscopy, preferably with differential interference contrast (DIC). Whenever possible, photographs and high-resolution videos of living animals should be used to later identify and describe internal taxonomic characters (*e.g.*, minute, thin-walled structures like the heart can easily collapse during preparation for histology and detection might be easier on living material than on histological sections). Radulae of meiofaunal species are resistant to fixation and preservation, but very small and structural details may not be adequately revealed using light microscopy requiring further analyses via scanning

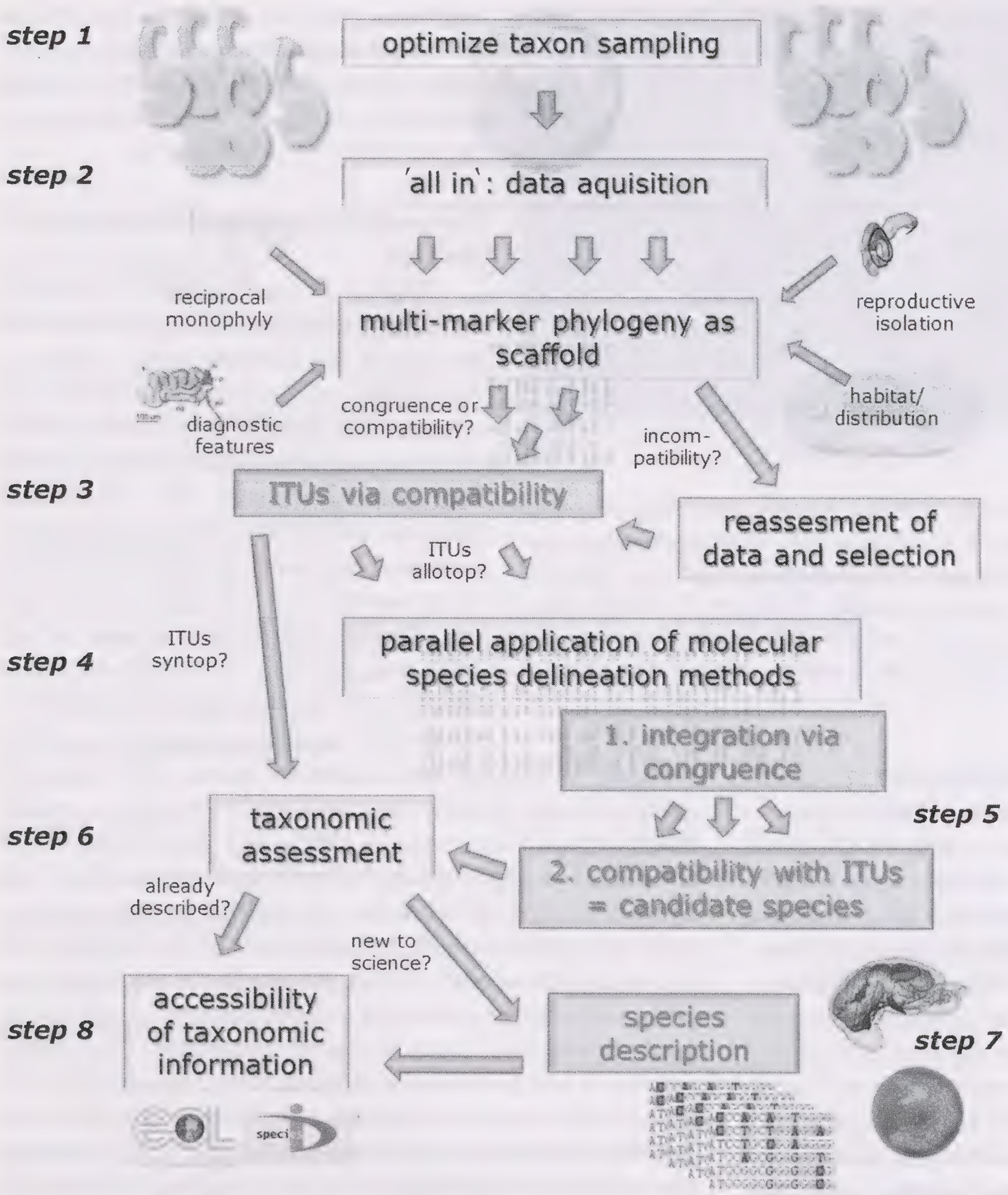

Figure 4. Flowchart on the proposed workflow on species delineation in elusive taxa, modified after the approach by Jörger *et. al.* (2012). (Color shown in electronic version only).

electron microscopy (SEM). Since preparation of minute radulae is challenging (Geiger *et al.* 2007), prior adequate documentation via light microscopy is indispensable. Rather than preparing whole mounted specimens for soft part anatomy, we recommend recovering the documented individuals and fix them for molecular studies or advanced morphological techniques (see Appendix 1, http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF) for different fixatives successfully applied).

A key to identify mesopsammic slugs

There are ongoing efforts to investigate in detail the microanatomical and morphological diversity of described mesopsammic lineages and their descendants and close relatives (*e.g.*, Neusser *et al.* 2006, 2007, Jörger *et al.* 2008, Rückert *et al.* 2008, Jörger *et al.* 2009, Neusser, Heß *et al.* 2009, Neusser, Martynov *et al.* 2009, Brenzinger, Wilson *et al.* 2011, Kohnert *et al.* 2011, Brenzinger, Haszprunar *et al.* 2013, Brenzinger, Padula *et al.* 2013, Kohnert *et al.* 2013, Jörger *et al.* 2014) and to reconstruct the evolution of phenotypes and biology from molecular approaches. This contributes to a better knowledge of the diversity in the mesopsammon and an evaluation of the diagnostic characters for identification of these taxa. As reviewed above, sampling efforts in the past years have discovered a series of species potentially new to science, which partially differ unequivocally from all described mesopsammic slug lineages by characters of the external morphology (see *e.g.*, Fig. 1A). On the other hand, the new material from the mesopsammon largely comprises cryptic lineages (especially within morphologically static Microhedylacea), which could only be revealed as novel evolutionary entities through the use of integrative approaches employing 3D-microanatomical descriptions and molecular data (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012, Jörger and Schrödl 2013), see discussion on species delimitation below. Nevertheless, many sea slug lineages inhabiting the interstitial—known and still unknown ones—may be tentatively identifiable to higher taxonomic categories such as family or genus-level in the field using the key presented herein (Appendix 3, http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF). For observing external features a dissecting microscope is necessary and internal features such as presence and structure of shell, spicules or radulae requires a transmission light microscope.

SPECIES DELIMITATION IN MEIOFAUNAL GASTROPODS – AND OTHER RARE AND ELUSIVE TAXA

Morphological species delineation

Traditionally, the taxonomy of gastropods relies on external morphological characters; even in 2006, approximately 80% of new gastropod species descriptions were based solely on shell characters combining the advantages of unproblematic preservation in natural history collections and potential for *post mortem* identification (Bouchet and Strong 2010). However, several studies with largely molecular scope have demonstrated the potentially high intraspecific variability of both shell morphology (*e.g.*, Hauswald *et al.* 2008, Bouchet and Strong 2010, Puillandre, Modica *et al.* 2012) and external characters in general, such as color variation in slugs (Nitz *et al.* 2009). When representatives of meiofaunal slug lineages were first discovered, their aberrant external morphology was in many cases sufficient for species delimitation (*e.g.*, Kowalevsky 1901a, 1901b). This changed with every new discovery of other closely related meiofaunal gastropods, making further characteristics of radulae and spicules obligatory for species delineation within clades (Salvini-Plawen 1973, Arnaud *et al.* 1986, Wawra 1987). In restricted geographic areas, a combination of these characters might still be sufficient to diagnose mesopsammic slugs (Eder *et al.* 2011), but on a broader scale these characters become insufficient (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012). External features in mesopsammic slugs are heavily constrained by the requirements of the spatially restricted habitat and provide little variation. Other features—such as the presence of externally visible eyes—show high intraspecific plasticity (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012).

For most gastropods the radula morphology is of major importance for taxonomy, in meiofaunal slugs diagnostic characters are, however, often limited to minute details (*e.g.*, the number of lateral denticles on the rhachidian tooth in Pseudovermidae (Urgorri *et al.* 1991)). Therefore, light-microscopic investigation might be insufficient to reliably analyze the minute radulae of meiofaunal gastropods and re-investigation by SEM is needed for reliable comparative analyses. Due to the partially minor interspecific variation, intraspecific and intraindividual variation requires special attention and in some cases radula characteristics may be insufficient to diagnose species (*e.g.*, Jörger *et al.* 2014).

Anatomical data included in species descriptions were traditionally based on morphological data from squeezed whole mounts (Kirsteuer 1973), whole mount or crush preparation of the radula (*e.g.*, Doe 1974) and/or the examination of histological sections of up to 10 µm thickness, which were frequently paraffin-based and distorted (*e.g.*, Odhner 1937, Marcus 1953, Challis 1968, Challis 1970, Morse 1976), and, therefore, not always reliable considering modern standards. Traditional manual reconstruction techniques from semithin histological sections (Sommerfeldt and Schrödl 2005) are time-consuming and challenging. We currently consider a 3D-based microanatomical approach most powerful: because modern 3D reconstructions based on µCT and synchrotron microtomography data currently do not allow for detailed microanatomical investigation in micromolluscs (Kunze 2013), we favor an approach using AMIRA software to reconstruct 3D models of all major organ systems based on serial semithin histological sections of 1–1.5 µm thickness (method after Ruthensteiner (2008); for methodological discussion see also DaCosta *et al.* (2007)).

Redescriptions of all major meiofaunal slug lineages based on advanced 3D-microanatomy in conjunction with ultrastructural data from, *e.g.*, sperm have compiled microanatomical characters across all organ systems, and these characters have proven reliable for taxonomic purposes (Neusser *et al.* 2006, Neusser and Schrödl 2007, Jörger *et al.* 2008, Neusser, Martynov *et al.* 2009, Jörger, Kristof *et al.* 2010, Martin *et al.* 2010, Brenzinger, Wilson *et al.* 2011, Eder *et al.* 2011, Kohnert *et al.* 2011, Brenzinger, Padula *et al.* 2013). These studies demonstrated the high quality of modern morphological approaches, which provide reliable, highly detailed diagnostic characters for taxonomic and systematic studies.

But even high-end morphological study reached its limits when confronted with the extraordinary degree of convergent adaptation that gastropods are notorious for (Ponder and Lindberg 1997, Dayrat and Tillier 2002, Wägele *et al.* 2014); and this adaptation is carried to an extreme in taxa that inhabit environments such as the mesopsammon, which selects for certain morphological and anatomical adaptations. Moreover, 3D-microanatomical approaches can be very time-consuming and taxonomists are faced with a trade-off between detailed accounts on a small number of specimens and estimations of the intraspecific vs. interspecific variability of characters. Therefore, in cases with ambiguous morphological data—which is the rule rather than the exception in mesopsammic slugs—only integrative approaches that combine evidence from morphology and molecules represent a viable method for tackling their diversity (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012). Given the putative high degree of cryptic speciation in meiofaunal taxa with supposedly low dispersal abilities (*e.g.*, Westheide and Schmidt 2003, Casu *et al.* 2009, Fontaneto *et al.* 2009, Leasi and Todaro 2009, Andrade *et al.* 2011, Jörger *et al.* 2012, Tulchinsky *et al.* 2012), it currently seems most efficient to reverse the traditional taxonomic workflows and initiate species delineation in meiofauna with barcoding and molecular species delineation approaches and integrate morphoanatomical and other data (rather than initiating with morphoanatomical lines of evidence and integrate molecular data).

Molecular species identification and delineation

DNA barcoding and molecular species delineation have been broadly advocated as fast and efficient means for dealing with the taxonomic impediment in times of biodiversity crisis (Blaxter *et al.* 2004, Blaxter *et al.* 2005, Hebert and Gregory 2005, Markmann and Tautz 2005, Hajibabaei *et al.* 2007). DNA-barcoding in its similarity-based form, which uses genetic distances, is a tool of species (re-)identification and not species discovery (DeSalle *et al.* 2005, DeSalle 2006). Lacking a predictive component, DNA-barcoding fails when no identical sequences are deposited in public databases (like Barcode of Life Data System http://www.boldsystems.org/ or GenBank http://www.ncbi.nlm.nih.gov/genbank/). Ongoing efforts of the workgroup include depositing barcodes of all valid mesopsammic slugs to public databases to allow for identification via barcodes (current coverage approx. 60%, partially still unpublished). However, as discussed above the vast majority of marine meiofauna have yet to be explored (Curini-Galletti *et al.* 2012), not to mention identified and sequenced, identical matches of newly collected material with deposited sequences will be the exception for meiofaunal taxa for decades to come (Jörger *et al.* 2012). Meiofaunal biodiversity assessments, therefore, will not be focused in typical DNA barcoding identification approaches, but require advanced methods of molecular species discovery.

Most of the numerous emerging programs and algorithms that have recently become available for molecular species delineation either rely on the comparison of genetic distances or use phylogenetic trees to estimate support under different model assumptions. To cluster sequences based on genetic distances, programs either use fixed or relative thresholds between intraspecific and interspecific variation (*e.g.*, Hebert *et al.* 2004, Jones *et al.* 2011, Ratnasingham and Hebert 2013) or aim to detect breaks in patterns of distance distribution, *i.e.*, a 'barcoding gap' (Meyer and Paulay 2005, Puillandre, Lambert *et al.* 2012). Distance-based approaches are usually based on mitochondrial cytochrome *c* oxidase subunit I (COI); this standard barcoding marker presents unique species-specific diagnostics in approximately 95% of all tested species. Moreover, the interspecific differences clearly exceed the intraspecific variability in most of these cases (Hebert, Cywinska *et al.* 2003, Hebert, Ratnasigham *et al.* 2003, Ratnasingham and Hebert 2013). Despite high success rates in praxis, the use of thresholds as proxy of species delimitation has been criticized for their arbitrariness; and criticism has been underscored by empirical studies demonstrating the disappearance or absence of a 'barcoding gap' (*i.e.*, intraspecific exceeds interspecific variability) with increased sample size (Moritz and Cicero 2004, Meyer and Paulay 2005, Wiemers and Fiedler 2007, Astrin *et al.* 2012). Model-based approaches such as the General-Mixed-Yule-Coalescent (GMYC) model infer evolutionary entities by evaluating likelihood values under speciation vs. population genetic processes on phylogenetic trees (Pons *et al.* 2006, Monaghan *et al.* 2009). But the accuracy of this and other model-based approaches also relies on sampling coverage (Lohse 2009). A dense sampling coverage is usually utopic when it comes to elusive taxa, whose sampling records frequently include a high degree of singletons. The rarity of taxa in undersampled datasets hampers reliable estimation of intraspecific vs. interspecific variation, and constitutes the primary obstacle of successful molecular species delineation in elusive taxa. Currently, a Bayesian approach evaluating for differences among gene trees is potentially best capable of dealing with singletons, provided that data from several independent markers are combined (Yang and Rannala 2010, Zhang *et al.* 2011). In the absence of a 'gold standard' for evaluating the performance of different species delineation approaches, and in view of high degrees of rarity in putatively undersampled datasets, an integrative approach of species delineation is needed for elusive taxa, one which allows for thorough cross-validation between approaches.

Workflow of integrative species delineation

Herein, we present a workflow capable of dealing with the above-mentioned problems, which are likely symptomatic for most meiofaunal taxa and other little explored and rare taxa such as, *e.g.*, many deep-sea clades, or many invertebrates in general. Due to the putative high degree of cryptic speciation and intraspecific variability on morphological character sets, the workflow is founded on molecular data. Faced with incomplete datasets and rarity, however, it is designed to make best use of the taxonomic information scattered across different lines of evidence. The proposed workflow (see Fig. 4) is based on the approach described in Jörger *et al.* (2012).

Step 1: Optimize taxon sampling and character sampling

In concordance with previous species delineation workflows (*e.g.*, Puillandre, Modica *et al.* 2012), this approach emphasizes the importance of dense taxon sampling to ensure reliable species delineation and to avoid overestimating interspecific variability (Hebert *et al.* 2004). In elusive taxa, taxonomists frequently lack knowledge on biology, dispersal abilities and geographic distribution, which requires an even stronger emphasis on targeted taxon sampling with regard to both geography and phylogenetic relationships. This includes collecting and analyzing several individuals from populations covering the potential geographic range of a taxon. Hence this workflow requires an *a priori* survey of the described species of a lineage; and whenever possible, material of valid species derived from type material or specimens re-collected at type localities should be included.

Under the unified species concept, species are defined as independently evolving metapopulation lineages, with the former secondary species criteria of competing concepts serving as equal operational criteria, *i.e.*, lines of evidence (de Queiroz 2005b, 2007). As central operational criteria serve intrinsic reproductive isolation, monophyly, exclusive coalescence, diagnosibility and deficits of genetic intermediates; the reliability of proposed species hypotheses increases with the number of supporting lines of evidence (de Queiroz 2005b, 2007). Entities discovered in molecular species delineation approaches should, therefore, be supported by a minimum of one line of evidence. Consequently, the workflow requires the grouping of all characters sets which were evaluated and selected by the taxonomist as contributions to one of these operational criteria (*e.g.*, molecular data, morphological and anatomical characters with special emphasis on reproductive features, geographic distribution, behavioral data, ecological niches). Therein, the workflow strongly encourages to initially give equal priority to all putatively useful character sets.

Step 2: 'All-in' – plotting of data onto a molecular phylogeny

Studies in species delineation of elusive taxa such as mesopsammic slugs should aim to gather as much putatively relevant information from as many different sources as possible (*i.e.*, morphoanatomical, ecological, molecular, and so on). Despite all efforts to compile 'complete' data matrices, in reality some populations will provide exhaustive information, while others will be represented by singletons only. Furthermore, amplification success may vary among samples, species and individuals, as bulk fixation of sediment samples usually degrades DNA. And finally, immature specimens can prevent the exploration of reproductive features and body retraction or damages often occur. In typical broad-scale barcoding approaches for biodiversity assessments, amplification problems of the COI-barcoding marker can result in incomplete, ambiguous sequences that are unable to pass quality filters on automated pipelines on Barcode of Life Data systems (BOLD) (Ratnasingham and Hebert 2013), thus, causing a potentially 'correct' lineage to be irretrievably lost for further assessments. To ensure the inclusion of all available lineages in this species delineation approach in spite of missing data, the scattered information on individual lines of evidence must be compensated by the amount of (self-contained) lines of evidence. Therefore, and to account for problems of incompatibility between species and gene trees (caused mainly by incomplete lineage sorting, pseudogenes, or introgression (Bensasson *et al.* 2001, Funk and Omland 2003, Song *et al.* 2008), this approach is based on 'multi-barcoding', including independently evolving markers (ideally from mitochondria and nucleus) (Jörger *et al.* 2012). As this approach is based on phylogenetic theory via the criterion of monophyly, single gene trees are calculated from each individual marker. The risk of producing artificial topologies is minimized by using Bayesian and/or Maximum Likelihood algorithms, rather than rapid distance based methods. Each gene should be checked for reticulate evolution (*e.g.*, using Dendroscope 3 (Huson and Scornavacca 2012)). Then, a concatenated all-marker phylogeny serves as scaffold for plotting other sources of data (focusing on those that putatively serve as operational criteria).

There are two major advantages of this unfortunately time-consuming initial step: 1) The unvalued objective plotting of the available characters of all terminals (or at least populations, if there is no doubt on conspecificity) in the dataset without biased pre-selection is based either on initial (single-gene) molecular data or on taxonomic intuition relying on, *e.g.*, morphological criteria. A prefiltering of available data into morphotypes (Riedel, Sagata, Suhardjono *et al.* 2013) leaves potential cryptic species undiscovered and is, therefore, not advisable for meiofaunal taxa. 2) The potential for quality checks and cross-validation between different lines of evidence. This critical reassessment of the primary data can help to reveal problematic molecular markers or potential homoplasies on morphological characters.

Step 3: 'Wild cards' and selection of integrative taxonomic units (ITUs) via compatibility.

Based on the plotted data, integrative taxonomic units (ITUs) are defined: Integrative taxonomy is commonly considered best practice (Dayrat 2005, Will *et al.* 2005, Valdecasas *et al.* 2008, Padial and de la Riva 2010, Padial *et al.* 2010, Astrin *et al.* 2012, Riedel, Sagata, Suhardjono *et al.* 2013), but approaches differ considerably in how exactly they integrate their data, with far-reaching consequences for the resulting species hypothesis. Typical large-scale barcoding workflows cluster COI sequences into operational taxonomic units (OTUs) based on genetic distances via different algorithms, and they encourage the addition of accessory data from other molecular markers or *e.g.*, morphology (Jones *et al.* 2011, Puillandre, Modica *et al.* 2012, Ratnasingham and Hebert 2013). When more data is added, the species diagnosis becomes integrative, whereas species delineation, which has led to the discovery of the OTU, remains based on a single line of evidence and will not be questioned or critically revised by additional data.

When truly integrating data into the process of species delineation, there is debate on the degree of congruence that different characters need to provide (Padial *et al.* 2010). Integrating via congruence requires a minimum of two selected lines of evidence to support the proposed species hypothesis, while in 'integration via cumulation' approaches the divergence of any character can justify the designation of species (Padial *et al.* 2010). The former approach promotes taxonomic stability, but implies the risk of underestimating species numbers. Integrative taxonomy via cumulation, on the other hand, tends to overestimate species, but is thereby likely best

suited to discover recent lineages (Padial *et al.* 2010). Ideally, ITUs can be selected across the integrative scaffold established in this workflow via congruence across all lines of evidence, *i.e.*, reciprocal monophyly supported by morphological features and geographic and habitat boundaries. For small datasets, concordance between operational criteria can be evaluated by eye, but especially in larger datasets, the use of statistical methods for testing of support values as developed by Cardoso *et al.* (2009) is advisable. The herein presented workflow suggests a less stringent application of congruence, promoting integration via compatibility, *i.e.*, allowing for entities supported by some and uncontradicted by other lines of evidence (see the 'minimum consensus' approach in Jörger *et al.* (2012)). This accommodates the fact that the process of speciation does not necessarily implement changes on all different levels of characters (Padial and de la Riva 2010, Padial *et al.* 2010) and that methods of detection have different sensitivities, but the approach remains conservative in relying solely on uncontradicted support for a particular species hypothesis.

The effects of speciation patterns may, however, result in incongruent datasets (Padial and de la Riva 2010, Padial *et al.* 2010). Integrative taxonomy, thus, must not be misunderstood as simply adding more and more data, rather it urges cautious selection of the appropriate character set for the species under investigation (Valdecasas *et al.* 2008). Faced with incongruence, the debate on which character set is best suited to species delineation cannot be solved universally, but has to be decided in each individual case with regard to the specifics of each taxon.

At this point the proposed workflow offers a potential short-cut to species assignment: reciprocally monophyletic clades occurring in syntopy, *i.e.*, in the same biotope, can be considered species under the unifying species concept, combining the operational criteria of intrinsic reproductive isolation with reciprocal monophyly (de Queiroz 2005a, 2007). The evaluation of syntopy in meiofauna is problematic, however, due to the largely unknown ecological interactions and potentially small-scale microhabitats.

Although less conservative than integrative taxonomy via (strict) congruence, the compatibility approach will nevertheless tend to lump species. Because it relies on the criterion of reciprocal monophyly, this initial step is likely not suited for detecting recently evolved lineages (Knowles and Carstens 2007, Sauer and Hausdorf 2012). It, therefore, needs to be refined in Step 5 in order to uncover any potential lumping of species.

Step 5: Parallel application of available methods of molecular species delineation

As discussed above, the accuracy of all available algorithms of molecular species delineation suffers from undersampled datasets and the inclusion of singletons (Jörger *et al.* 2012) and also tends to oversplit datasets in empirical studies (*e.g.*, Sauer and Hausdorf 2012). In the absence of a 'gold-standard' for comparing the performance of each analysis on the respective dataset, our workflow suggests an unbiased parallel application of available molecular species delineation methods across all markers. Special emphasis should be given to model-based approaches such as GMYC (Pons *et al.* 2006, Monaghan *et al.* 2009)—provided that minimum requirements, *e.g.*, on sampling density, are fulfilled—and algorithms capable of dealing with rarity, such as Birky's (2013) simple coalescence theory based approach. The inference of genetic connectivity via haplotype networks applying statistical parsimony (Clement *et al.* 2000) is merely an indirect method of estimating species boundaries (Pons *et al.* 2006); nevertheless, it visualizes the genetic structure in the dataset, which is valuable for cross-validating molecular entities revealed by other approaches. Even though the performance of distance-based approaches is conceptually disputed and practically hindered in lineages that putatively suffer from incomplete sampling (Meyer and Paulay 2005, Hickerson *et al.* 2006, Meier *et al.* 2006, Wiemers and Fiedler 2007, Meier *et al.* 2008), the parallel application of Refined Single Linkage analysis (RESL) (Ratnasingham and Hebert 2013) or the Automatic Barcode Gap Discovery (ABGD) (Puillandre, Lambert *et al.* 2012) contributes to the empirical evaluation of the efficiency of standard barcoding approaches on elusive taxa. All the above methods are applied to single genes, and single gene histories may differ and, thus, results need to be compared and their significance evaluated. Using concatenated markers is not appropriate since information from single loci may be dominant and mask potential conflict. A powerful approach based on multilocus markers is the Bayesian Species delineation (Yang and Rannala 2010, Zhang *et al.* 2011); the more independent markers are available for combination, the better it can deal with rarity of samples.

Step 6: Congruence in molecular species delineation and compatibility of ITUs to determine candidate species

To exploit the potential of molecular species delineation methods for revealing prior lumping of species, integration via compatibility is inapplicable in this step, as it would directly transfer over-splitting of each individual method to the identification of the candidate species. The workflow aims for a cross-validation between the different approaches achieved by integrating the results via congruence. Only molecular entities supported by all molecular species delineation approaches are then further integrated via compatibility to the formerly identified ITUs, in order to lead to the final determination of the candidate species.

Step 7: Assessment of the taxonomic history

With regard to ecological studies or biodiversity assessments, an advantage of rare or elusive taxa compared to common or

hyperdiverse clades is the fact that the former usually lack an exhaustive history of available descriptions and putative synonyms, with the majority of lineages being still undescribed. To avoid the creation of synonyms, it is crucial to clarify whether the discovered candidate species already bears a valid name, *i.e.*, a thorough literature review on all potential names available. If several names are available, the oldest name has priority according to nomenclatural rules. If old descriptions do not permit unambiguous assignment, we recommend using the oldest name that can be reliably assigned to the newly delimitated species (Ornelas-Gatdula *et al.* 2012). Alternatives would be to resurrect unused or dubious older names, which is a rather arbitrary procedure, or to deliberately establish and name a "new" species despite the existence of already available names, thus, creating a junior synonym.

Step 8: Species description

Independently evolving lineages discovered as candidate species, but which cannot be assigned to valid species, should be described to receive formal recognition. Molecular species delineation approaches frequently terminate their efforts with the discovery of independently evolving lineages (*e.g.*, Fontaneto *et al.* 2009, Monaghan *et al.* 2009, Astrin *et al.* 2012). The BIN system (Barcode Index Numbering on BOLD) even propagates the use of OTUs as an alternative taxonomic reference system (Ratnasingham and Hebert 2013), drawing on initial proposals on DNA taxonomy (Tautz *et al.* 2003). The amount of deposited sequences, which are unidentified at the species level and bear other unique identifiers, has grown tremendously in the course of the barcoding endeavor over the past few years (see http://iphylo.blogspot.de/2011/04/dark-taxa-genbank-in-post-taxonomic.html). Clustering sequences into OTUs may be sufficient for further applications such as biodiversity assessments, while unidentified sequences can still contribute valuable information in the absence of species assignment. The name of a species is entirely extrinsic, it could, thus, be replaced by any alternative identifier such as a BIN. However, the use of OTUs not merely as a source of taxonomic characters, but also as a taxonomic reference system, can be problematic when it comes to establishing novel unique identifiers, as these identifiers create parallel taxonomies flagged with new acronyms and classification systems in competition with traditional taxonomy (Jörger and Schrödl 2013). The Linnaean name anchors the species to its taxonomic history and all available biological and morphological data (Polaszek *et al.* 2008, Patterson *et al.* 2010). Moreover, the genus name includes a hypothesis on phylogenetic relationships. A species name can be linked to life science identifiers via ZooBank (http://zoobank.org/), capable of uniquely linking content on this species through different computational platforms (Polaszek *et al.* 2008). In order to reduce and not enhance impediments in taxonomy by parallel yet inconsistent identifiers, discovered lineages should be connected to the taxonomic history of a clade by providing formal descriptions (Jörger and Schrödl 2013). Depending on the chosen operational criteria of species delimitation, this set of characters will form the basis for the diagnoses of species. Jörger and Schrödl (2013) illustrated how molecular diagnostic characters can be extracted via character-based barcoding approaches (Sarkar *et al.* 2008, Bergmann *et al.* 2009) and used for taxonomic description. Future efforts should aim to automate the extraction of diagnostic molecular characters to facilitate and accelerate species description, as has already been achieved in 'turbo-taxonomic' approaches for morphological data (Butcher *et al.* 2012, Riedel, Sagata, Suhardjono *et al.* 2013, Riedel, Sagata, Surbakti *et al.* 2013). However, the fair option to diagnose species entirely based on molecular characters, morphological data should nevertheless be included in the species description (Jörger and Schrödl 2013).

Step 9: Ensure accessibility of all data

Digital technologies provide powerful methods for making taxonomic data more accessible to the research communities via, *e.g.*, virtual access to museum collections, digitalized biodiversity libraries, online registration systems for zoological names and infrastructure for biogeographic assessments (*e.g.*, Wheeler 2008, Padial and de la Riva 2010, Padial *et al.* 2010). Next to the obligatory voucher deposition in museum collections (including vouchers of extracted DNA) and of genetic sequences in public databases, data from species descriptions can now be deposited in online platforms (*e.g.*, the Encyclopedia of Life http://eol.org/). This increases the accessibility of taxonomic knowledge and allows for dynamic expansion of species diagnoses through future studies (Riedel, Sagata, Suhardjono *et al.* 2013), ideal for gradually augmenting knowledge on enigmatic taxa.

CONCLUSIONS AND OUTLOOK

The marine mesopsammon is one of the largest, yet least explored habitats on Earth, and the taxonomic deficit is correspondingly high. To date, the mesopsammic fauna has been largely neglected in conservation approaches, despite their doubtless important role, *e.g.*, in the marine food web. The comparably low reproductive output and the poor dispersal abilities of mesopsammic slugs indicate small ranges of distribution and high degrees of endemism. The threat to their diversity by habitat destruction is consequently high. By providing practical sampling instructions on how to explore the mesopsammic malacofauna in the field, we aim to encourage the inclusion of this fauna into biodiversity assessments. A boost in sampling efforts world-wide—but with special emphasis to the numerous unsampled tropical regions—is urgently needed to get reliable estimations on the diversity of

these enigmatic taxa still hidden in global sands. Currently, the micromorphological diversity of known microslugs is comparatively investigated and the inclusion of minute mesopsammic sea slugs into multi-locus analyses on Heterobranchia has demonstrated how theses enigmas can help to understand the phylogenetic relationships and evolution of larger clades. We now need to fill in the gaps in existing taxon samplings with remaining elusive taxa (*e.g.*, Pseudovermidae) in order to supplement the complex picture of heterobranch evolution step by step and to understand the evolutionary pathways which led into the mesopsammon.

We emphasize that next to the initial exploration in the field, there is also a theoretical debate needed on how to efficiently and reliably delineate meiofaunal species—a task which usually presents a struggle with rarity and uniformity. Despite all valuable advances in accelerating the rates of taxonomic descriptions to face the 'taxonomic impediment' in times of biodiversity crisis, here we promote a form of 'deep taxonomy' in cases where the evolutionary history of species requires a thorough integrative workflow. This will ensure that these small clades do not slip through automated 'turbo-taxonomy' pipelines.

ACKNOWLEDGMENTS

We thank Dr. Philippe Bouchet (MNHN, Paris, France) for the invitation to present this topic at the "Mollusks: Magnitude of molluscan diversity – the known and the unknown" Symposium of the 78th Annual Meeting of the American Malacological Society and to contribute to this special volume. We are indebted to Dr. Ira Richling (SMNS, Stuttgart, Germany) for her infinite patience in compiling the proceedings. We are grateful to Nerida Wilson, Alexander Martynov, and numerous friends and colleagues who share our interest in meiofaunal research and provided us with valuable material of mesopsammic slugs from all over the world. Christina Flammensbeck helped in compiling the data on Pseudovermidae. KMJ and MS thank the VW Stiftung and the German Research Foundation (DFG SCHR667/4 and 13), respectively, for funding our research. The GeoBioCenter LMU kindly provided an AMIRA license and diving equipment. We wish to express our gratitude to Victoriano Urgorri and an anonymous referee for helpful comments on the manuscript.

LITERATURE CITED

Andrade, S. C. S., J. L. Norenburg, and V. N. Solferini. 2011. Worms without borders: genetic diversity patterns in four Brazilian Ototyphlonemertes species (Nemertea, Hoplonemertea). *Marine Biology* **158**: 2109–2124.

Arnaud, P. M., C. Poizat, and L. v. Salvini-Plawen. 1986. Marine-interstitial Gastropoda (including one freshwater interstitial species). *In*: L. Botosaneanu, ed., *Stygofauna Mundi.* Brill/Backhuys, Leiden, Netherlands. Pp. 153–161.

Astrin, J. J., P. E. Stuben, B. Misof, J. W. Wagele, F. Gimnich, M. J. Raupach, and D. Ahrens. 2012. Exploring diversity in cryptorhynchine weevils (Coleoptera) using distance-, character- and tree-based species delineation. *Molecular Phylogenetics and Evolution* **63**: 1–14.

Ax, P. 1969. Populationsdynamik, Lebenszyklen und Fortpflanzungsbiologie der Mikrofauna des Meeressandes. *Zoologischer Anzeiger* **32, Suppl.**: 66–113. [In German].

Bensasson, D., D. X. Zhang, D. L. Hartl, and G. M. Hewitt. 2001. Mitochondrial pseudogenes: Evolution's misplaced witnesses. *Trends in Ecology & Evolution* **16**: 314–321.

Bergmann, T., H. Hadrys, G. Breves, and B. Schierwater. 2009. Character-based DNA barcoding: a superior tool for species classification. *Berliner und Münchener Tierärztliche Wochenschrift* **122**: 446–450.

Birky, C. W., Jr. 2013. Species detection and identification in sexual organisms using population genetic theory and DNA Sequences. *PLoS ONE* **8**: e52544. doi: 10.1371/journal.pone.0052544.

Blaxter, M., B. Elsworth, and J. Daub. 2004. DNA taxonomy of a neglected animal phylum: An unexpected diversity of tardigrades. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**: S189–S192.

Blaxter, M., J. Mann, T. Chapman, F. Thomas, C. Whitton, R. Floyd, and E. Abebe. 2005. Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**: 1935–1943.

Botosaneanu, L. 1986. *Stygofauna mundi*. Brill/Backhuys, Leiden, Netherlands.

Bouchet, P. and E. E. Strong. 2010. Historical name-bearing types in marine molluscs: An impediment to biodiversity studies? *In*: A. Polaszek, ed., *Systema Naturae.* CRC Press, London. Pp. 63–74.

Brenzinger, B., G. Haszprunar, and M. Schrödl. 2013. At the limits of a successful body plan – 3D microanatomy, histology and evolution of *Helminthope* (Mollusca: Heterobranchia: Rhodopemorpha), the most worm-like gastropod. *Frontiers in Zoology* **10**: 37.

Brenzinger, B., T. P. Neusser, K. M. Jörger, and M. Schrödl, M. 2011. Integrating 3D microanatomy and molecules: Natural history of the Pacific freshwater slug *Strubellia* Odhner, 1937 (Heterobranchia, Acochlidia), with description of a new species. *Journal of Molluscan Studies* **77**: 351–374.

Brenzinger, B., V. Padula, and M. Schrödl. 2013. Insemination by a kiss? Interactive 3D-microanatomy, biology and systematics of the mesopsammic cephalaspidean sea slug *Pluscula cuica* Marcus, 1953 from Brazil (Gastropoda: Euopisthobranchia: Philinoglossidae). *Organisms Diversity & Evolution* **13**: 33–54.

Brenzinger, B., N. G. Wilson, and M. Schrödl. 2011. 3D microanatomy of a gastropod 'worm', *Rhodope rousei* n. sp. from Southern Australia. *Journal of Molluscan Studies* **77**: 375–387.

Brenzinger, B., N. G. Wilson, and M. Schrödl. 2014. Microanatomy of shelled *Koloonella cf. minutissima* (Laseron, 1951) (Gastropoda: 'Lower' Heterobranchia: Murchisonellidae) does not contradict a sister-group relationship with enigmatic Rhodopemorpha slugs. *Journal of Molluscan Studies*: doi:10.1093/mollus/eyu036.

Butcher, B. A., M. A. Smith, M. J. Sharkey, and D. L. J. Quicke. 2012. A turbo-taxonomic study of Thai *Aleiodes* (*Aleiodes*) and *Aleiodes* (*Arcaleiodes*) (Hymenoptera: Braconidae: Rogadinae) based largely on COI barcoded specimens, with rapid descriptions of 179 new species. *Zootaxa* **3457**: 1–232.

Cardoso, A., A. Serrano, and A. P. Vogler. 2009. Morphological and molecular variation in tiger beetles of the *Cicindela hybrida* complex: is an 'integrative taxonomy' possible? *Molecular Ecology* **18**: 648–664.

Casu, M., T. Lai, D. Sanna, P. Cossu, and M. Curini-Galletti. 2009. An integrative approach to the taxonomy of the pigmented European *Pseudomonocelis* Meixner, 1943 (Platyhelminthes: Proseriata). *Biological Journal of the Linnean Society* **98**: 907–922.

Challis, D. A. 1968. A new genus and species of the order Acochlidiacea (Mollusca: Opisthobranchia) from Melanesia. *Transactions of the Royal Society New Zealand (Zoology)* **10**: 191–197.

Challis, D. A. 1969. *Philine exigua* n. sp. (Opisthobranchia: Bullomorpha), a minute interstitial species from Melanesia. *Transactions of the Royal Society New Zealand Biological Sciences* **11**: 177–186.

Challis, D. A. 1970. *Hedylopsis cornuta* and *Microhedyle verrucosa*, two new Acochlidiacea (Mollusca: Opisthobranchia) from the Solomon Islands Protectorate. *Transactions of the Royal Society New Zealand Biological Sciences* **12**: 29–40.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: A computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.

Coull, B. C. and O. Giere. 1988. The history of Meiofauna. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 14–17.

Curini-Galletti, M., T. Artois, V. Delogu, W. H. De Smet, D. Fontaneto, U. Jondelius, F. Leasi, A. Martínez, I. Meyer-Wachsmuth, K. S. Nilsson, P. Tongiorgi, K. Worsaae, and M. A. Todaro. 2012. Patterns of diversity in soft-bodied meiofauna: Dispersal ability and body size matter. *PLoS ONE* **7**: e33801. doi: 10.1371/journal.pone.0033801.

Da Costa, S., C. M. Cunha, L. R. L. Simone, and M. Schrödl. 2007. Computer-based 3-dimensional reconstruction of major organ systems of a new aeolid nudibranch subspecies, *Flabellina engeli lucianae*, from Brazil (Gastropoda : Opisthobranchia). *Journal of Molluscan Studies* **73**: 339–353.

Dayrat, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**: 407–415.

Dayrat, B. and S. Tillier. 2002. Evolutionary relationships of euthyneuran gastropods (Mollusca): a cladistic re-evaluation of morphological characters. *Zoological Journal of the Linnean Society* **135**: 403–470.

de Queiroz, K. 2005a. Different species problems and their resolution. *Bioessays* **27**: 1263–1269.

de Queiroz, K. 2005b. A unified concept of species and its consequences for the future of taxonomy. *Proceedings of the California Academy of Sciences* **56**: 196–215.

de Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**: 879–886.

DeSalle, R. 2006. Species discovery versus species identification in DNA barcoding efforts: Response to Rubinoff. *Conservation Biology* **20**: 1545–1547.

DeSalle, R., M. G. Egan, and M. Siddall. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**: 1905–1916.

Doe, D. A. 1974. A new species of the Order Acochlidiacea (Opisthobranchia: Microhedylidae) from New England. *Transactions of the American Microscopical Society* **93**: 241–247.

Eder, B., M. Schrödl, and K. M. Jörger, 2011. Systematics and redescription of the European meiofaunal slug *Microhedyle glandulifera* (Kowalevsky, 1901) (Heterobranchia: Acochlidia): evidence from molecules and morphology. *Journal of Molluscan Studies* **77**: 388–400.

Fontaneto, D., M. Kaya, E. A. Herniou, and T. G. Barraclough, 2009. Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution* **53**: 182–189.

Fukuda, H. and R. Ueshima. 2010. Smeagolidae of Japan – a marine pulmonate family newly discovered from the northern hemisphere. *Tropical Natural History* **Suppl. 3**: 334 [abstract].

Funk, D. J. and K. E. Omland. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics* **34**: 397–423.

Garcia-Alvarez, O., V. Urgorri, and F. J. Cristobo. 2000. Synopsis of the intersitial Solenogastres (Mollusca). *Argonauta* **14**: 27–37.

Geiger, D. L., B. A. Marshall, W. F. Ponder, T. Sasaki, and A. Warén, A. 2007. Techniques for collecting, handling, preparing, storing and examining small molluscan specimens. *Molluscan Research* **27**: 1–45.

Giard, A. 1904. Sur une faunule caractéristique des sables à Diatomées d'Ambleteuse (Pas-de-Calais). *Cahier Royale de la Societe Biologic Paris* **56**: 107–165. [In French].

Giere, O. 2009. *Meiobenthology: The microscopic motile fauna of aquatic sediments*. Springer Verlag, Berlin, Germany.

Giere, O., A. Eleftheriou, and D. J. Murison. 1988. Abiotic factors. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 61–78.

Hajibabaei, M., G. A. C. Singer, P. D. N. Hebert, and D. A. Hickey. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* **23**: 167–172.

Haszprunar, G. 1985. The Heterobranchia - a new concept of the phylogeny of the higher Gastropoda. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* **23**: 15–37.

Haszprunar, G. and M. Heß. 2005. A new *Rhodope* from the Roscoff area (Bretagne), with a review of *Rhodope* species. *Spixiana* **28**: 193–197.

Hauswald, A.-K., C. Albrecht, and T. Wilke. 2008. Testing two contrasting evolutionary patterns in ancient lakes: Species flock versus species scatter in valvatid gastropods of Lake Ohrid. *Hydrobiologia* **615**: 169–179.

Hebert, P. D. N. and T. R. Gregory. 2005. The promise of DNA barcoding for taxonomy. *Systematic Biology* **54**: 852–859.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: S96–S99.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 313–321.

Hebert, P. D. N., M. Y. Stoeckle, T. S. Zemlak, and C. M. Francis. 2004. Identification of birds through DNA barcodes. *PLoS Biology* **2**: 1657–1663.

Hickerson, M. J., C. P. Meyer, and C. Moritz. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* **55**: 729–739.

Higgins, R. P. and H. Thiel. 1988a. *Introduction to the study of Meiofauna*. Smithsonian Institution Press, Washington, D.C., U.S.A.

Higgins, R. P. and H. Thiel. 1988b. Prospectus. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 11–13.

Huson, D. H. and C. Scornavacca. 2012. Dendroscope 3: An interactive tool for rooted phylogenetic trees and networks. *Systematic Biology* **61**: 1061–1067.

Jones, M., A. Ghoorah, and M. Blaxter. 2011. jMOTU and Taxonerator: Turning DNA barcode sequences into annotated operational taxonomic units. *PLoS ONE* **6**: e19259. doi: 10.1371/journal.pone.0019259.

Jörger, K. M. and M. Schrödl. 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology* **10**: 59. doi:10.1186/1742-9994-10-59.

Jörger, K. M., T. P. Neusser, G. Haszprunar, and M. Schrödl. 2008. Undersized and underestimated: 3D-visualization of the Mediterranean interstitial acochlidian gastropod *Pontohedyle milaschewitchii* (Kowalevsky, 1901). *Organisms, Diversity & Evolution* **8**: 194–214.

Jörger, K. M., M. Heß, T. P. Neusser, and M. Schrödl. 2009. Sex in the beach: spermatophores, dermal insemination and 3D sperm ultrastructure of the aphallic mesopsammic *Pontohedyle milaschewitchii* (Acochlidia, Opisthobranchia, Gastropoda). *Marine Biology* **156**: 1159–1170.

Jörger, K. M., A. Kristof, A. Klussmann-Kolb, and M. Schrödl. 2010. Redescription of the meiofaunal gastropod *Parhedyle cryptophthalma*, with focus on nervous system and sensory organs (Acochlidia, Panpulmonata). *Spixiana* **33**: 161–170.

Jörger, K. M., J. L. Norenburg, N. G. Wilson, and M. Schrödl. 2012. Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. *BMC Evolutionary Biology* **12**: 245. doi:10.1186/1471-2148-12-245.

Jörger, K. M., T. Stoschek, A. E. Migotto, G. Haszprunar, and T. P. Neusser. 2014. 3D-microanatomy of the mesopsammic *Pseudovermis salamandrops* Marcus, 1953 from Brazil (Nudibranchia, Gastropoda). *Marine Biodiversity*. doi: 10.1007/s12526-014-0224-5.

Jörger, K. M., I. Stöger, Y. Kano, H. Fukuda, T. Knebelsberger, and M. Schrödl. 2010. On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. *BMC Evolutionary Biology* **10**: 323. doi:10.1186/1471-2148-10-323.

Kirsteuer, E. 1973. Occurrence of the interstitial Opisthobranch *Unela remanei* Marcus, in the Caribbean Sea. *Mitteilungen Institute Colombo-Aleman Investigationes Cientificas* **7**: 41–46.

Klussmann-Kolb, A., A. Dinapoli, K. Kuhn, B. Streit, and C. Albrecht. 2008. From sea to land and beyond--new insights into the evolution of euthyneuran Gastropoda (Mollusca). *BMC Evolutionary Biology* **8**: 57. doi:10.1186/1471-2148-8-57.

Knowles, L. L. and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* **56**: 887–895.

Kohnert, P., T. P. Neusser, K. M. Jörger, and M. Schrödl. 2011. Time for sex change! 3D-reconstruction of the copulatory system of the 'aphallic' *Hedylopsis ballantinei* (Gastropoda, Acochlidia). *Thalassas* **27**: 113–119.

Kohnert, P., B. Brenzinger, K. Jensen, and M. Schrödl, M. 2013. 3D-microanatomy of the semiterrestrial slug *Gascoignella aprica* Jensen, 1985—a basal plakobranchacean sacoglossan (Gastropoda, Panpulmonata). *Organisms Diversity & Evolution* **13**: 583–603.

Kowalevsky, A. 1901a. Études anatomiques sur le genre *Pseudovermis*. *Memoires de l'Academie Imperiale des Sciences de St.Petersbourg* **12**: 1–28. [In French].

Kowalevsky, A. 1901b. Les Hédylidés, étude anatomique. *Memoires de l'Academie Imperiale des Sciences de St.Petersbourg* **12**: 1–32. [In French].

Kunze, T. 2013. *Comparative 3D Microanatomy and Systematics of Skeneimorph Gastropods, with a Survey on Epipodial Tentacles in Lower Gastropods*. Ph.D. Thesis. *Department Biology II*. Ludwig-Maximilians-Universität München, Munich, Germany.

Leasi, F. and M. Todaro. 2009. Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). *Marine Biology* **156**: 1335–1346.

Lohse, K. 2009. Can mtDNA barcodes be used to delimit species? A Response to Pons *et al.* (2006). *Systematic Biology* **58**: 439–441.

Lovén, S. 1844. Chaetoderma, ett nytt maskslägte n.g. *Öfversigt af Kungl Vetenskaps-Akademien Förhandlingar* **1**: 116+pl. 112. [In Swedish].

Marcus, E. 1953. Three Brazilian sand-Opisthobranchia. *Boletim de Faculdade de Filosofia, Ciencias e Letras de Universidade de Sao Paulo, Zoology* **164**: 165–203.

Markmann, M. and D. Tautz. 2005. Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**: 1917–1924.

Martin, R., K.-H. Tomaschko, M. Heß, and M. Schrödl. 2010. Cnidosac-related structures in *Embletonia* (Mollusca, Nudibranchia) compared with dendronotacean and aeolidacean species. *The Open Marine Biology Journal* **4**: 96–100.

Martynov, A. 2007. *Embletonia pulchra* (Alder & Hancock, 1844). In: Sea Slug Forum Australian Museum, Sydney. Available from http://seaslugforum.net/factsheet/emblpulc.

Meier, R., G. Zhang, and F. Ali. 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. *Systematic Biology* **57**: 809–813.

Meier, R., K. Shiyang, G. Vaidya, and P. K. L. Ng. 2006. DNA barcoding and taxonomy in diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715–728.

Meyer, C. P. and G. Paulay. 2005. DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* **3**: 2229–2238.

Miller, M. C. and R. C. Willan. 1992. Redescribtion of *Embletonia gracile* Risbec, 1928 (Nudibranchia, Embletoniidae): Relocation to suborder Dendronotacea with taxonomic and phylogenetic implications. *Journal of Molluscan Studies* **58**: 1–12.

Milne Edwards, H. 1848. Note sur la classification naturelle des mollusques gasteropodes. *Annales des Sciences Naturelles, Zoologie* **3**: 102–112. [In French].

Monaghan, M. T., R. Wild, M. Elliot, T. Fujisawa, M. Balke, D. J. Inward, D. C. Lees, R. Ranaivosolo, P. Eggleton, T. G. Barraclough, and A. P. Vogler. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* **58**: 298–311.

Moritz, C. and C. Cicero. 2004. DNA barcoding: Promise and pitfalls. *PLoS Biology* **2**: 1529–1531.

Morse, M. P. 1976. *Hedylopsis riseri* sp. n., a new interstitial mollusc from the New England Coast (Opisthobranchia, Acochlidiacea). *Zoologica Scripta* **5**: 221–229.

Morse, M. P. and A. H. Scheltema. 1988. Aplacophora. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 447–450.

Neusser, T. P. 2011. Marine interstitial *In*: P. Bouchet, H. Le Guyaderand, and O. Pascal, eds., *The Natural History of Santo.* (Patrimoines naturels; 70), MNHN, Paris; IRD, Marseille; PNI, Paris. Pp. 572.

Neusser, T. P. and M. Schrödl. 2007. *Tantulum elegans* reloaded: a computer-based 3D-visualization of the anatomy of a Caribbean freshwater acochlidian gastropod. *Invertebrate Biology* **126**: 18–39.

Neusser, T. P., H. Fukuda, K. M. Jörger, Y. Kano, and M. Schrödl. 2011. Sacoglossa or Acochlidia? 3D reconstruction, molecular phylogeny and evolution of Aitengidae (Gastropoda: Heterobranchia). *Journal of Molluscan Studies* **77**: 332–350.

Neusser, T. P., M. Heß, and M. Schrödl, M. 2009. Tiny but complex - interactive 3D visualization of the interstitial acochlidian gastropod *Pseudunela cornuta* (Challis, 1970). *Frontiers in Zoology* **6**: 20.

Neusser, T. P., M. Heß, G. Haszprunar, and M. Schrödl, M. 2006. Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acochlidian gastropod from Bermuda. *Journal of Morphology* **267**: 231–247.

Neusser, T. P., M. Heß, G. Haszprunar, and M. Schrödl. 2007. Sperm ultrastructure of *Microhedyle remanei*, an interstitial acochlidian gastropod with dermal fertilization. *Journal of the Marine Biological Association of the United Kingdom* **87**: 747–754.

Neusser, T. P., K. M. Jörger, and M. Schrödl. 2011. Cryptic species in tropic sands - Interactive 3D anatomy, molecular phylogeny and evolution of meiofaunal Pseudunelidae (Gastropoda, Acochlidia). *PLoS ONE* **6**: e23313. doi: 10.1371/journal.pone.0023313.

Neusser, T. P., A. V. Martynov, and M. Schrödl. 2009. Heartless and primitive? 3D reconstruction of the polar acochlidian gastropod *Asperspina murmanica*. *Acta Zoologica (Stockholm)* **90**: 228–245.

Neusser, T. P. and M. Schrödl, M. 2009. Between Vanuatu tides: 3D anatomical reconstruction of a new brackish water acochlidian gastropod from Espiritu Santo. *Zoosystema* **31**: 453–469.

Nicholls, A. G. 1935. Copepods from the interstitial fauna of a sandy beach. *Journal of the Marine Biological Association of the United Kingdom* **20**: 379–405.

Nitz, B., R. Heim, U. E. Schneppat, I. Hyman, and G. Haszprunar. 2009. Towards a new standard in slug species descriptions: the case of *Limax sarnensis* Heim & Nitz n. sp. (Pulmonata: Limacidae) from the Western Central Alps. *Journal of Molluscan Studies* **75**: 279–294.

Odhner, N. 1937. *Hedylopsis suecica* n. sp. und die Nacktschneckengruppe Acochlidiacea (Hedylacea). *Zoologischer Anzeiger* **120:**51–64. [In German].

Ornelas-Gatdula, E., Y. Camacho-García, M. Schrödl, V. Padula, Y. Hooker, T. M. Gosliner, and Á. Valdés. 2012. Molecular systematics of the '*Navanax aenigmaticus*' species complex (Mollusca, Cephalaspidea): coming full circle. *Zoologica Scripta* **41**: 374–385.

Padial, J. M. and I. de la Riva. 2010. A response to recent proposals for integrative taxonomy. *Biological Journal of the Linnean Society* **101**: 747–756.

Padial, J. M., A. Miralles, I. de la Riva, and M. Vences. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7**: 16. doi:10.1186/1742-9994-7-16.

Patterson, D. J., J. Cooper, P. M. Kirk, R. L. Pyle, and D. P. Remsen P. 2010. Names are key to the big new biology. *Trends in Ecology & Evolution* **25**: 686–691.

Pfannkuche, O. and H. Thiel. 1988. Sample processing. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 134–145.

Poizat, C. 1984. Seasonal variations of Mediterranean interstitial opisthobranch assemblages. *Hydrobiologia* **118**: 83–94.

Poizat, C. 1991. New data on an interstitial opisthobranch assemblage and other meiofauna from the Skagerrak, Sweden. *Journal of Molluscan Studies* **57**: 167–177.

Poizat, C. and P. M. Arnaud. 1988. Gastropoda and Bivalvia. *In*: R. P. Higgins and H. Thiel, eds., *Introduction to the study of Meiofauna.* Smithsonian Institution Press, Washington, D.C., U.S.A.. Pp. 451–456.

Polaszek, A., R. L. Pyle, and D. Yanega. 2008. Animal names for all: ICZN, ZooBank and the new taxonomy. *In*: Q. Wheeler, ed., *The New Taxonomy.* CRC Press, London, Great Britain. Pp. 129–142.

Ponder, W. F. and D. R. Lindberg. 1997. Towards a phylogeny of gastropod molluscs: Analysis using morphological characters. *Zoological Journal of the Linnean Society* **119**: 83–265.

Pons, J., T. G. Barraclough, J. Gomez-Zurita, A. Cardoso, D. P. Duran, S. Hazell, S. Kamoun, W. D. Sumlin, and A. P. Vogler. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55**: 595–609.

Puillandre, N., A. Lambert, S. Brouillet, and G. Achaz. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21**: 1864–1877.

Puillandre, N., M. V. Modica, Y. Zhang, L. Sirovich, M. C. Boisselier, C. Cruaud, M. Holford, and S. Samadi. 2012. Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology* **21**: 2671–2691.

Ratnasingham, S. and P. D. N. Hebert. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS ONE* **8**: e66213. doi: 10.1371/journal.pone.0066213.

Remane, A. 1940. Einführung in die zoologische Ökologie der Nord- und Ostsee. *In*: G. Grimpe and E. Wagler, eds., *Die Tierwelt der Nord- und Ostsee.* Geest & Portig, Leipzig, Germany. Pp. 1–238. [In German].

Remane, A. 1952. Die Besiedlung des Sandbodens im Meer und die Bedeutung der Lebensformtypen für die Ökologie. *Verhandlungen der Deutschen Zoologischen Gesellschaft*: 327–339. [In German].

Riedel, A., K. Sagata, Y. Suhardjono, R. Tänzler, and M. Balke. 2013. Integrative taxonomy on the fast track - towards more sustainability in biodiversity research. *Frontiers in Zoology* **10**: 15. doi:10.1186/1742-9994-10-15.

Riedel, A., K. Sagata, S. Surbakti, R. Tänzler, and M. Balke. 2013. One hundred and one new species of *Trigonopterus* weevils from New Guinea. *Zookeys*: 1–150.

Rückert, I., A. Altnöder, and M. Schrödl. 2008. Computer-based 3D anatomical reconstruction and systematic placement of the mesopsammic gastropod *Platyhedyle denudata* Salvini-Plawen, 1973 (Opisthobranchia, Sacoglossa). *Organisms, Diversity & Evolution* **8**: 358–367.

Rundell, R. J. and B. S. Leander. 2010. Masters of miniaturization: Convergent evolution among interstitial eukaryotes. *Bioessays* **32**: 430–437.

Ruthensteiner, B. 2008. Soft Part 3D visualization by serial sectioning and computer reconstruction. *Zoosymposia* **1**: 63–100.

Salvini-Plawen, L. v. 1968. Neue Formen im marinen Mesopsammon: Kamptozoa und Aculifera (nebst der für die Adria neuen Sandfauna). *Annalen des Naturhistorischen Museums in Wien A* **72**: 231–272. [In German].

Salvini-Plawen, L. v. 1973. Zur Kenntnis der Philinoglossacea und der Acochlidiacea mit Platyhedylidae fam. nov. (Gastropoda, Cephalaspidea). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* **11**: 110–133. [In German].

Sarkar, I. N., P. J. Planet, and R. DeSalle. 2008. CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources* **8**: 1256–1259.

Sauer, J. and B. Hausdorf. 2012. A comparison of DNA-based methods for delimiting species in a Cretan land snail radiation reveals shortcomings of exclusively molecular taxonomy. *Cladistics* **28**: 300–316.

Schrödl, M. 2006. Techniques for collecting interstitial opisthobranchs. In: Sea Slug Forum Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/inteextr

Schrödl, M. and T. P. Neusser. 2010. Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society* **158**: 124–154.

Schrödl, M., K. M. Jörger, A. Klussmann-Kolb, and N. G. Wilson. 2011. Bye bye "Opisthobranchia"! A review on the contribution of mesopsammic sea slugs to euthyneuran systematics. *Thalassas* **27**: 101–112.

Sommerfeldt, N. and M. Schrödl. 2005. Microanatomy of *Hedylopsis ballantinei*, a new interstitial acochlidian gastropod from the Red Sea, and its significance for phylogeny. *Journal of Molluscan Studies* **71**: 153–165.

Song, H., J. E. Buhay, M. F. Whiting, and K. Crandall. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 13486–13491.

Swedmark, B. 1964. The interstitial fauna of marine sand. *Biological Reviews* **39**: 1–42.

Swedmark, B. 1968. The biology of interstitial Mollusca. *Symposium of the Zoological Society of London* **22**: 135–149.

Tautz, D., P. Arctander, A. Minelli, R. H. Thomas, and A. P. Vogler. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* **18**: 70–74.

Tillier, S. and W. F. Ponder. 1992. New species of *Smeagol* from Australia and New Zealand, with a discussion of the affinities of the genus (Gastropoda, Pulmonata). *Journal of Molluscan Studies* **58:** 135–155.

Tulchinsky, A., J. Norenburg, and J. Turbeville. 2012. Phylogeography of the marine interstitial nemertean *Ototyphlonemertes parmula* (Nemertea, Hoplonemertea) reveals cryptic diversity and high dispersal potential. *Marine Biology* **159**: 661–674.

Urgorri, V., F. Cobo, and C. Besteiro. 1991. *Pseudovermis artabrensis* (Nudibranchia, Aeolidoidea), a new species from Galicia, Spain. *Journal of Molluscan Studies* **57**: 189–197.

Valdecasas, A. G., D. Williams, and Q. D. Wheeler. 2008. 'Integrative taxonomy' then and now: a response to Dayrat (2005). *Biological Journal of the Linnean Society* **93**: 211–216.

Vonnemann, V., M. Schrödl, A. Klussmann-Kolb, and H. Wägele. 2005. Reconstruction of the phylogeny of the Opisthobranchia (Mollusca: Gastropoda) by means of 18S and 28S rRNA gene sequences. *Journal of Molluscan Studies* **71**: 113–125.

Wägele, H., Klussmann-Kolb, A., Verbeek, E., and Schrödl, M. 2014. Flashback and foreshadowing—a review of the taxon Opisthobranchia. *Organisms Diversity & Evolution* **14**: 133–149.

Wawra, E. 1974. The rediscovery of *Strubellia paradoxa* (Strubell) (Gastropoda: Euthyneura: Acochlidiacea) on the Solomon Islands. *The Veliger* **17**: 8–10.

Wawra, E. 1979. *Acochlidium sutteri* nov. spec. (Gastropoda, Opisthobranchia, Acochlidiacea) von Sumba, Indonesien. *Annalen des Naturhistorischen Museums in Wien. Serie B. Botanik und Zoologie* **82**: 595–604. [In German].

Wawra, E. 1987. Zur Anatomie einiger Acochlidia (Gastropoda, Opisthobranchia) mit einer vorläufigen Revision des Systems und einem Anhang über Platyhedylidae (Opisthobranchia, Ascoglossa). Wien: Universität Wien. [In German].

Westheide, W. 1987. Progenesis as a principle in meiofauna evolution. *Journal of Natural History* **21**: 843–854.

Westheide, W. and H. Schmidt. 2003. Cosmopolitan versus cryptic meiofaunal polychaete species: An approach to a molecular taxonomy. *Helgoland Marine Research* **57**: 1–6.

Wheeler, Q. 2008. Introductory: Toward the new taxonomy. *In*: Q. Wheeler (Ed) *The new taxonomy.* CRC Press, London, Great Britain. Pp. 1–18.

Wiemers, M. and K. Fiedler. 2007. Does the DNA barcoding gap exist? A case study in blue butterflies (Lepidoptera: Lycaenidae). *Frontiers in Zoology* **4**: 8. doi:10.1186/1742-9994-4-8.

Will, K. W., B. D. Mishler, and Q. D. Wheeler. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54**: 844–851.

Wilson, N. G., K. M. Jörger, and M. Schrödl. 2010. Reducing an enigma: placing the vermiform Rhodopemorpha (Gastropoda) in a phylogeny. *In*: S. Panha, C. Sutcharitand, and P. Tongkerd, eds., *17th World Congress of Malacology* (abstract, p. 37). Tropical Natural History, Phuket, Thailand.

Worsaae, K. and R. M. Kristensen. 2005. Evolution of interstitial Polychaeta (Annelida). *Hydrobiologia* **535**: 319–340.

Worsaae, K., W. Sterrer, S. Kaul-Strehlow, A. Hay-Schmidt, and G. Giribet. 2012. An anatomical description of a miniaturized acorn worm (Hemichordata, Enteropneusta) with asexual reproduction by paratomy. *PLoS ONE* **7:** e48529. doi: 10.1371/journal.pone.0048529.

Yang, Z. H. and B. Rannala. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 9264–9269.

Zhang, C., D.-X. Zhang, T. Zhu, and Z. Yang. 2011. Evaluation of a Bayesian coalescent method of species delimitation. *Systematic Biology* **60**: 747–761.

Received: 08 February 2014**; accepted:** 27 May 2014**; final revisions received:** 15 July 2014